Howley, P. M., Yang, Y.-C., Spalholz, B. A., and Kanda, M. S.

1986. Papillomaviruses. Wiley, Chichester (Ciba Foundation Symp. no. 120), pp. 30–52.

Other support: Damon Runyon-Walter Winchell Cancer Fund Fellowship and NRSA Fellowship Grant.

From the Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD.

THE E5 TRANSFORMING GENE OF BOVINE PAPILLOMAVIRUS ENCODES A SMALL, HYDROPHOBIC POLYPEPTIDE.

Bovine papillomavirus contains two independent transforming genes that have been mapped to the E5 and E6 open reading frames (ORFs). The E5 transforming protein was identified by means of an antiserum against a synthetic peptide corresponding to the 20 COOH-terminal amino acids of the E5 ORF. The E5 polypeptide is the smallest viral transforming protein yet characterized. It had an apparent size of 7 kilodaltons. The transforming polypeptide is encoded entirely within the second half of the E5 ORF and its predicted amino acid composition is very unusual: 68% of the amino acids are strongly hydrophobic and 34% are leucine. Cell fractionation studies localized this polypeptide predominantly to cellular membranes.

Schlegel, R., Wade-Glass, M., *Rabson, M. S.*, and Yang, Y.-C.

Science 233(4764): 464–467, July 25, 1986.

From the Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD.

THE NEW BIOMEDICAL TECHNOLOGY.

New methods for studying the genetic information of humans in health and disease are emerging from basic science laboratories. Because these approaches are yielding fundamental insights for diagnosing and treating disease, it is important that practitioners begin to understand these methods and how they are used. Methods for genetic [illegible] recombinant DNA techniques consist of isolation, separation, propagation in bacteria or animals, and molecular hybridization of DNA. The study of RNA allows determination of gene expression. These methods are being used to understand cancer, identify hereditary illness, produce pharmaceuticals, and diagnose common clinical problems, such as infectious diseases.

Sanders, C. H.

The Western Journal of Medicine **143**: 819–824, 1985.

Other support: National Institutes of Health and the Eleanor Roosevelt Institute for Cancer Research.

From the Eleanor Roosevelt Institute for Cancer Research and the Department of Medicine, University of Colorado Health Sciences Center, Denver.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

A METHOD FOR ISOLATING NICK-TRANSLATED DNA BY SUBSEQUENT SEPARATION ON LOW-MELTING-TEMPERATURE AGAROSE

Nick-translation of cloned DNA segments followed by separation on low-melting-temperature agarose has been used to obtain multiple radiolabeled DNA probes from a single nick-translation procedure. This technique avoids premature inhibition of enzymatic reactions. Examples of the utility of the procedure are presented and the advantages and drawbacks are discussed.

Lyon, E., S. Fisher, J. H. et al.

Analytical Biochemistry **140**: 25–29, 1984.

Other support: National Institutes of Health, American Lung Association and Bonfils-Roosevelt Institute for Cancer Research.

From the Webb-Waring Institute and Department of Biochemistry, Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center and Rose Medical Center, Denver.

PURIFICATION OF HUMAN DNA (CYTOSINE-5-)-METHYLTRANSFERASE

We have developed a facile procedure for the purification of DNA methyltransferase activity from human placenta. The procedure avoids the isolation of nuclei and the dialysis and chromatography of large volumes. A purification of 38,000-fold from the whole cell extract has been achieved. The procedure employs ion exchange, affinity, and hydrophobic interaction chromatography coupled with preparative glycerol-gradient centrifugation. A protein of 126,000 daltons was found to correlate with the activity and was the major band seen in the most highly purified material after SDS gel electrophoresis. This observation, coupled with an observed sedimentation coefficient of 5.8S, suggests that the enzyme is composed of a single polypeptide chain of this molecular weight. Hemimethylated DNA was found to be the preferred substrate for the enzyme at each stage in the purification. The ratio of the activity of the purified product on hemimethylated to that on unmethylated M13 duplex DNA was about 12 to 1. Thus, the purified enzyme has the properties postulated for a maintenance methyltransferase. The availability of highly purified human DNA methyltransferase should facilitate the study of its structure, function, and expression in normal as well as neoplastic cells in both normal and transformed states.

Zweller, K. E. Rep[illegible] et al.

Journal of Cellular Biochemistry **29**: 37[illegible], 1985.

Other support: [illegible]

From the Department of [illegible] and Beckman Research Institute of the City of Hope, Duarte, CA.

CONCERTED DEGRADATION OF MIDDLE REPETITIVE SEQUENCES

The mechanism by which [illegible] patterns [illegible] during [illegible] formed in DNA [illegible] DNA [illegible]

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

Purified bacteriophage strains do not show clear sequence specificity [illegible] those from mouse have been shown to prefer cytosine residues in human methylated 5'CG dimers. These findings notwithstanding, Sano and Sager (1982) have reported that each of the repeating elements of bovine satellite DNA is thought to display a complex pattern of methylation within a given tissue, and that [illegible] of a restriction of the 5'CCGG and 5'TCGA tetramers are preferentially modified. Experiments discussed in this report suggest that molecular mechanisms exist that produce correlated alterations in the methylation state of broadly interspersed [illegible] DNA sequences.

See also S. Sano and Bolberg, M. E.

Biochemistry and Biophysics of DNA Methylation, Academic Press, 1985, Alan R. Liss, Inc., pp. 11-22.

Other support. National Institutes of General Medical Sciences.

From the Beckman Research Institute of the City of Hope, Department of Biological Sciences, Duarte, CA.

THE TUMORIGENICITY OF 5-AZACYTIDINE IN THE MALE LIVER RAT

5-Azacytidine was administered to young adult male Fischer 344 rats [illegible] to determine whether the [illegible] Starting at 6 and 8 weeks of age, groups of rats received the experimental [illegible] Several rats had multiple primary tumors. [illegible] was found for complete carcinogenicity in a variety of tumor types. [illegible] and [illegible] and [illegible] and

cell [illegible] and stable differentiation induced by BMS.

Tolberg, M. E., and Smith, S. S.

FEBS Letters 176(2):280-284, October 1984.

Other support: National Institutes of Health.

From the Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, Ca.

STRUCTURAL ORGANIZATION OF INTERSPERSED REPETITIVE ELEMENTS PRESENT IN THE DNA OF *MUS MUSCULUS*

Hybridization displays of the restriction fragments from the DNA of *Mus musculus* revealed a complex species specific pattern produced from nonsatellite repetitive sequences. The patterns have been used as a guide in the direct purification of a group of broadly interspersed repeated DNA sequences characterized by a 135? bp [illegible] that have been studied by molecular cloning, restriction mapping and genomic Southern blotting. These studies show that the cloned representatives originate from an abundant group of sequences that share homology with about 2% of the mouse genome. The sequences do not appear to share homology with mouse interspersed family-1 (MIF-1) nor with the major AT-rich satellite sequences of mouse. They appear to be part of a group of larger repetitive elements that is both broadly interspersed and heavily methylated in normal mouse tissue.

Tolberg, M. E., and *Smith, S. S.*

Biochimica et Biophysica Acta **783**:272-282, 1984.

Other support: National Institutes of Health.

From the Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA.

SELECTIVE PHOSPHORYLATION OF HUMAN DNA METHYLTRANSFERASE BY PROTEIN KINASE C

Human DNA methyltransferase, the enzyme thought to be responsible for the somatic inheritance of patterns of DNA methylation, is an effective substrate for phosphorylation by protein kinase C. This provides a plausible mechanistic link between the action of tumor promoting phorbol esters, which stimulate protein kinase C, and [illegible] patterns of DNA methylation often observed in transformed cells.

DePaoli Roach, A., Roach, P. J., Zucker, K. E., and *Smith, S. S.*

FEBS Letters 197(1,2):139-143, March 1986.

Other support: National Institutes of Health.

From the Departments of Enzymatic Research and Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

SUPPRESSION OF TUMORIGENICITY WITH CONTINUED EXPRESSION OF THE c-Ha-*ras* ONCOGENE IN EJ/BLADDER CARCINOMA-HUMAN FIBROBLAST HYBRID CELLS

A human tumor cell line (EJ) expressing an activated c-Ha-*ras* oncogene was fused with a normal human fibroblast cell line. This fusion resulted in hybrids that behaved as transformed cells in culture but failed to form tumors in nude (athymic) mice. After repeated cell passage, two tumorigenic segregants of the hybrids arose in culture. The levels of expression of activated c-Ha-*ras* mRNA and its protein product, p21, were similar in the EJ cell line, the nontumorigenic hybrids, and the tumorigenic segregants. DNA transfections of the hybrids were performed with activated c-Ha-*ras* plasmid constructs, and transfectants expressing a 2-fold level of c-Ha-*ras* relative to the hybrid cells were found to maintain the nontumorigenic phenotype. We suggest that expression of the active c-Ha *ras* oncogene is insufficient for the malignant transformation of these human cells.

Gusella, J. F., Der, C. J., Marshall, C. J., *Stanbridge, E. J.*

Proceedings of the National Academy of Sciences, USA **83**:5209–5213, July 1986.

Other support: National Cancer Institute and the Philip and Clarisse Hay Fund.

From the Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine.

SELECTIVE TRANSFER OF INDIVIDUAL HUMAN CHROMOSOMES TO RECIPIENT CELLS

Two hypoxanthine phosphoribosyltransferase-deficient human cell lines, D98-AH-2 and HT1080 6TG, were stably transfected with pSV2 *gpt*, a plasmid containing the selectable marker *Escherichia coli* xanthine-guanine phosphoribosyl transferase (Eco *gpt*). Hypoxanthine-aminopterin-thymidine-resistant transformants arose with a frequency of ca. 10^{-6} and contained mostly single, but occasional multiple, copies of the plasmid sequences. These transformants actively express the Eco *gpt* marker. Single chromosomes from two different HT1080 *gpt* transformants and one D98 *gpt* transformant, containing the integrated plasmid sequences, were transferred via microcell-mediated chromosome transfer to hypoxanthine phosphoribosyl transferase-deficient mouse A9 cells. The transferred human chromosomes were identified as 2, 4 and 22 by using a combination of G-11 staining, G-banding, isoenzyme analysis, and *in situ* hybridization. This system is being used to create a library of interspecies microcell hybrid clones each containing a unique single human chromosome in a mouse background. The complete library will represent the entire human karyotype.

Saxon, P. J., Srivatsan, E. S., Deipzig, G. V., Sameshima, J. H., and *Stanbridge, E. J.*

Molecular and Cellular Biology, 5:1430–1436, 1985.

Other support: National Cancer Institute and National Institutes of Health.

From the Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine.

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

PHAGOCYTES AS CARCINOGENS: MUTATIONS IN *ras* ONCOGENES PRODUCED BY HUMAN NEUTROPHILS

In a study of the relation between chronic inflammation and cancer, we exposed C3H mouse fibroblasts of the JOE 1-2 clone 8 line (JOE 1-2 cells) to human neutrophils stimulated to synthesize reactive oxygen intermediates with a cell-free enzymatic system generating superoxide anion from xanthine oxidase plus hypoxanthine. After exposure, the JOE 1-2 cells were either placed in tissue culture or immediately injected into athymic nude mice. Both malignant and benign tumors developed in mice that were injected with treated cells, but not in those injected with control cells. Tumor-bearing cells grown from benign tumor subsequently developed malignant properties in culture. Malignant transformation was also observed in fibroblasts exposed to [illegible]

Watanabe, S. S., Weinberg, A. F., Clark, E. P., and Stossel, T. P.

Science **227**, 412–414, 1985.

Other support. American Cancer Society, National Institutes of Health, Bristol-Myers, and the Edwin Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital Cancer Center, and Department of Medicine, Harvard Medical School, Boston.

AUGMENTED EXPRESSION OF NORMAL *c-mos* IS SUFFICIENT FOR COTRANSFORMATION OF RAT EMBRYO CELLS WITH A MUTANT *ras* GENE

The authors studied the effect of altered *c-mos* structure and expression upon the ability of *c-mos* and *ras* to cotransform normal rat embryo cells when it was supplemented by Ecotropic murine mutant cellular *ras* genes from LL-124 Harvey sarcoma cells. We studied cellular *c-mos* alleles cloned from normal and tumor tissues of chicken and human origin and found that only LL-*mos*, derived from a bursal lymphoma in which an avian leukosis virus long terminal repeat resides within the first *c-mos* intron, in either transcriptional orientation, had cotransforming activity. No activity was observed with normal chicken and human *c-mos* alleles, two other "activated" chicken *c-mos* alleles (LL-*mos*$_{tr}$ and LL-*mos*$_{ts}$), and two human *c-mos* genes (HSR-*mos* and HDM-*mos*) from human promyelocytic and fibroblast cell lines. COLO-320 cells, which have *c-mos* amplified. Some of these inactive alleles had the following alterations that are frequently found in tumor-derived *c-mos*: point mutations affecting the encoded protein (LL-*mos*$_{tr}$), a truncated structure with loss of the first normal splice site (LL-*mos*$_{ts}$), and IDM-*mos*, an "provirus" integrated within intron 1 of the *c-mos* gene (LL-*mos*$_{tr}$, LL-*mos*$_{ts}$, and IDM-*mos*). The following experimental results implicated transcriptional activity as directly related to the transforming activity observed for these alleles: (1) whether cotransfected into Rat-2 cells, LL-*mos* was the only oncogene that the other inactive alleles and our antigenically expressed HSR-*mos*, IDM-*mos*, or normal human or normal chicken *c-mos* replaced with the transcriptional control of retroviral long terminal repeats or increased expression of normal human *c-mos* under the in-

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

fluence of a retroviral enhancer element was accompanied by cellular oncogene activation in vivo. We concluded that augmented expression of even an normal c-myc gene is sufficient for cotransforming activity and that additional structural alterations [illegible] tumor-derived alleles are neither necessary nor sufficient for the gene's malignant or embryonal cell cotransforming properties.

Lee, W. H., Schwab, M., Westaway, D., and Varmus, H. E.

Molecular and Cellular Biology 5(12):3345-3356, 1985.

Other support: The George Hooper Research Foundation, National Institutes of Health and American Cancer Society.

From the Department of Medicine, Cancer Research Institute and Howard Hughes Medical Institute Laboratory, Department of Microbiology and Immunology, University of California, San Francisco.

A DI-GLUCURONIDATION INHIBITOR REDUCES THE INDUCTION BY BENZO(a)PYRENE OF A 60-KILODALTON ONCOFETAL PROTEIN AND DNA-BINDING *IN VIVO*

The objective of the present study was to evaluate the possibility of reducing carcinogenicity of benzo(a)pyrene (BP) in rats by the use of 2,5-di-O-acetyl-D-glucaro 1,4:6,3-dilactone (DAGDL). Our previous study demonstrated that DAGDL, a precursor of D-glucaro 1,4-lactone, known to be a natural inhibitor of β-glucuronidase, caused a 70% reduction in the incidence of mammary tumor induction in rats by 7,12-dimethylbenz(a)anthracene (DMBA). Coadministration of DAGDL also reduced the induction of a 60-Kd oncofetal protein shown to be associated with carcinogenesis and tumorigenesis, and markedly reduced binding of DMBA to organ DNA. In the present study, we have examined the effect of DAGDL coadministration on the induction of the oncofetal protein and its binding to organ DNA in vivo. DAGDL suppressed by 25% the induction of the oncofetal protein. By a carcinogenic dose of BP, DAGDL also reduced BP binding to organ DNA, i.e. by 50-70%, depending on the organ examined. DAGDL appears to act as an anti-carcinogen depending on the organ examined. DAGDL appears to act as an anti-carcinogen by inhibiting deglucuronidation of hydroxylated metabolites of PAH, thereby increasing detoxification and carcinogenic clearance.

Walaszek, Z., Hanausek-Walaszek, M., and Webb, T. E.

In: Cooke, M. W. and Dennis, A. J. (eds.): *Polynuclear Aromatic Hydrocarbons: Ninth International Symposium on Chemistry, Characterization and Carcinogenesis*, Columbus, OH: Battelle Press, 1984, pp. 947-959.

Other support: National Cancer Institute.

From the Department of Physiological Chemistry and The Comprehensive Cancer Center, Ohio State University, Columbus.

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DIETARY GLUCARATE MEDIATED REDUCTION OF SENSITIVITY OF MURINE STRAINS TO CHEMICAL CARCINOGENESIS

Serum β-glucuronidase activity is shown to differ quantitatively in the following strains of mice, listed in order of increasing activity: C3H, C57BL/6, BALB/c, DBA/2, ICR/+, SENCAR, A/He. The level of the enzyme in the murine strains is shown to correlate with the urinary excretion of 17-ketosteroids, which in turn reflects the endogenous level of androgens. Dietary calcium D-glucarate, an *in vivo* β-glucuronidase inhibitor, reduced the steady state level of both β-glucuronidase and 17-ketosteroid excretion in the high-sensitivity strains, A/He and SENCAR, to that of strains known to be resistant to chemical carcinogenesis. Sensitivity of the A/He strain is significantly reduced by dietary calcium glucarate, which is shown to increase DNA binding of [^{3}H]-benzo[a]pyrene by a factor of 2. Pharmacokinetic data are provided by benzo[a]pyrene

Walaszek, Z., et al.

Cancer Letters 33:259-267, 1986

Other support: National Cancer Institute

From the Department of Physiological Chemistry and the Comprehensive Cancer Center, The Ohio State University, Columbus

USE OF SHORT DNA OLIGONUCLEOTIDES FOR DETERMINATION OF DNA SEQUENCE MODIFICATIONS INDUCED BY BENZO[a]PYRENE DIOL EPOXIDE

Various organic agents that alkylate DNA are known to induce mutations in bacterial and mammalian cells. The process, nature and location of modified DNA sequences in such mutants are often difficult to ascertain. In this report, a 10 base-pair oligomer (*Bam*HI linker) is treated with the *trans* benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide and inserted into replicative form DNA of phage M13 by ligation at a specific restriction site. *Escherichia coli* are transfected with the recombinant DNA containing the alkylated target; progeny viral plaques are selected, and their DNAs are subjected to DNA sequence analysis at the region of oligomer insertion. For the alkylated inserts used in this study, the DNA sequence analysis of progeny viral DNA showed that nucleotide deletions were present in every clone examined. These deletions occurred primarily, but not exclusively, at G+C cluster regions, varied from 1 to 28 base pairs in length, and involved both target and non-target nucleotides. A second type of repair, which restores most of the original nucleotide bases in the alkylated insert, is also implied by the DNA sequence data obtained.

Wei, S.-J. C., Desai, S. M., Harvey, R. G., and *Weinstein, I. B.*

Proceedings of the National Academy of Sciences, USA 81:5639-5643, 1984

Other support: U. S. Department of Energy, American Cancer Society and National Cancer Institute

From the Franklin McLean Memorial Research Institute, the Department of Biochemistry and the Ben May Laboratory for Cancer Research, University of Chicago

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BACTERIA ASSOCIATED WITH OBSTRUCTIVE PULMONARY DISEASE ELABORATE EXTRACELLULAR PRODUCTS THAT STIMULATE MUCIN SECRETION BY EXPLANTS OF GUINEA PIG AIRWAYS

Cell-free filtrates from broth cultures of *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Streptococcus pneumoniae* stimulate secretion of sulfated glycoconjugates by explants of guinea pig trachea. The stimulatory effect is not related to toxicity or damage to the respiratory mucosa, as well as could be determined by ultrastructural examination of the explants after exposure. Bacteria isolated from patients with a history of chronic obstructive lung disease (*P. aeruginosa* from cystic fibrosis, *H. influenzae* and *S. pneumoniae* from chronic bronchitis) do not demonstrate increased frequency of positive strains or greater stimulation of secretion than organisms isolated from other individuals. At least three stimulatory substances are found in cell-free filtrates of *P. aeruginosa*. They appear to be proteins of molecular weight 60,000–100,000 as determined by gel filtration. Within the crude filtrate, they are relatively stable to heat, proteolysis, and storage at 4°C and in liquid nitrogen. The stimulatory activity is not lost upon subculture of the bacteria. When isolated from the filtrate by column chromatography, they become labile to heat and trypsin. Isolated active fractions show proteolytic activity coinciding with mucin-stimulating capacity, suggesting a relationship with *Pseudomonas* proteases.

Stimulatory substances released by *S. pneumoniae* and *H. influenzae* appear to be different from those elaborated by *Pseudomonas*. They are extremely labile to heat and storage, and the capacity to stimulate secretion is lost on subculture. Preliminary gel filtration indicates the *S. pneumoniae* stimulatory substance(s) is in a molecular weight range of 100,000–300,000 daltons, while that of *H. influenzae* is between 50,000 and 200,000. The results suggest bacteria which chronically infect or colonize respiratory airways of individuals suffering from obstructive lung disease can elaborate extracellular product(s) capable of stimulating secretion of mucin. Thus, the bacteria themselves may contribute to local manifestations and, ultimately, to the pathogenesis of obstructive disease.

Adler, K. B. et al.

American Journal of Pathology **125**:501-514, 1986.

Other support: National Institutes of Health.

From the Departments of Pathology and Medicine, College of Medicine, University of Vermont, Burlington.

PREVENTIVE THERAPY OF EMPHYSEMA: LESSONS FROM THE ELASTASE MODEL

In 1964, Gross and colleagues described the first reproducible model of pulmonary emphysema produced in experimental animals by intratracheal installation of the plant protease, papain. These and many subsequent experiments have shown that

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[illegible] proteases [illegible] [illegible] [illegible]

Other support. California Research Inc., Medical Education Fund of the American Lung Association of California, and Research Evaluation and Allocation Committee of the School of Medicine, University of California, San Francisco.

From the Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco.

GROWTH RATE OF PERIVASCULAR CUFFS IN LIQUID-INFLATED DOG LUNG LOBES

In the early stages of pulmonary edema, excess liquid leaving the pulmonary exchange vessels accumulates in the peribronchovascular interstitium, where it forms large peribronchovascular cuffs. The peribronchovascular interstitium therefore acts as a reservoir to protect the air spaces from alveolar flooding. The rate of liquid accumulation and the liquid storage capacity of the cuffs determine how quickly alveolar flooding is likely to follow once edema formation has begun. To measure the rate and capacity of interstitial filling we inflated 11 isolated degassed dog lung lobes with liquid to an inflation pressure of 14 cmH$_2$O (total lung capacity) for 1–300 min, then froze the lobes in liquid N$_2$. We made photographs of 20 randomly selected 12 × 8 mm cross sections from each lobe and measured cuff volume from the photographs by point counting. We found that cuff volume increased from 2.2% of air-space volume after 1 min of inflation to 9.3% after 300 min. To measure the driving pressure responsible for cuff formation we used micropipettes to measure subpleural interstitial liquid pressure at the hilum of three additional lobes. With liquid inflation pressure set to 18 cmH$_2$O, interstitial pressure rose exponentially to 11.5 cmH$_2$O. Interstitial compliance calculated from our volume and pressure measurements equaled 0.069 ml/cmH$_2$O·g wet wt^{-1}, a value similar to that measured in air-inflated lungs. Goldberg [*Am. J. Physiol.* 239 (*Heart Circ. Physiol.* 8): H189–H198, 1980] has likened interstitial filling to the charging of a capacitor, a process that follows a mono-exponential time course. A best fit mono-exponential through our cuff volume data had a time constant of 56 min, which is three times longer than that reported previously. We conclude that the previously reported time constant measured filling of only the portion of the peribronchovascular interstitium closest to the exchange vessels.

Correspondence K. L.

Journal of Applied Physiology **61**: 647-653, 1986.

This work was supported by the California Research and Medical Education Fund of the American Lung Association of California, and the Research Evaluation and Allocation Committee of the School of Medicine, University of California, San Francisco.

From the Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco.

AN *IN VITRO* MODEL FOR POLYMORPHONUCLEAR LEUKOCYTE-INDUCED INJURY TO AN EXTRACELLULAR MATRIX

Neutrophil (PMN) adherence to endothelial cells stimulated by inflammatory mediators is believed to be the initial step in the sequence leading to subsequent extravascular accumulation of fluid and protein. Here

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we describe the interaction between activated human polymorphonuclear leukocytes (PMN) and basement membranes of the amnion. Isolated human PMN were placed on the stromal and basement membrane sides of the amnion. PMN were separately exposed to the PMN-conditioned medium, phorbol myristate acetate (PMA), or the chemotactic peptide fMLP. After the cells were activated, the PMN suspension was removed and centrifuged, and the supernatant was assayed for superoxide anion (O_2^-) and for elastase activity. Injury to the basement membrane was evaluated by transmission electron microscopy and by measurement of hydroxyproline (HP) released from the membrane matrix. Although both stimuli induced comparable oxidant-dependent release of O_2^-, only PMA stimulated elastase release. The HP effects were similar when either the stromal or the basement membrane side was exposed to PMN. PMA-stimulated cells and supernatants from PMA-stimulated cells caused solubilization of matrix HP after different incubation times. Electron microscopy confirmed the disruption of the basement membrane of the amnion by PMA-stimulated PMN. Oxidant scavengers (SOD and catalase) did not prevent matrix degradation, and elastase inhibitors by a specific chloromethylketone inhibitor diminished HP release on both sides of the amnion by activated PMN supernatants, but only at the basement membrane side by intact PMN. We conclude that in this model, elastase rather than oxygen radicals solubilizes HP from the matrix.

Sibille, Y., Reynolds, H. Y., Mukaida, N., Matsushima, K., Polonski, P., and Gee, J. B. L.

American Review of Respiratory Disease, 134 (3): 434–440, 1986.

Other support: National Institutes of Health.

From the Pulmonary Section, Department of Medicine, Yale University School of Medicine, New Haven, CT.

INFLUENCE OF SPUTUM IgA AND ELASTASE ON TRACHEAL CELL BACTERIAL ADHERENCE

Bacterial adherence to respiratory epithelium is the mechanism of airway colonization, but the influence of airway proteins on this phenomenon is largely unknown. We measured tracheal cell-bacterial binding in 16 subjects with chronic tracheostomy and related these results to measurements of sputum IgA, [illegible] and bacterial protein from the same subjects. Tracheal cell adherence was related directly to sputum elastase activity ($r = 0.69$, $p < 0.02$) and elastase activity, primarily neutrophil protease, was higher in subjects colonized by *Pseudomonas aeruginosa* than in those without this finding ($p < 0.05$). Sputum levels of IgA-binding protein were related inversely to tracheal cell adherence ($r = -0.64$, $p < 0.02$). Sputum IgA concentrations, however, were unaffected by host nutritional status and airway elastase activity. Evidence that elastase can degrade sputum IgA was provided by an inverse relationship between these 2 proteins ($r = -0.85$, $p < 0.02$) and by an in vitro experiment showing augmentation of IgA by pretreatment with neutrophil elastase. Bacterial protease digestion and separation indicated IgA fragmentation after 1 h of incubation. These data suggest that, once adherence has been established, the resulting inflammatory response may

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

foster microbial growth by an elastase-dependent IgA cleavage and hence ciliated tracheal cell adherence.

Niederman, M. S., Merrill, W. W., Polonski, L. M., and *Gee, J. B. L.*

American Review of Respiratory Disease 133:255-260, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Pulmonary Section, Department of Medicine, Yale University School of Medicine, New Haven, CT.

BORDETELLA PERTUSSIS TRACHEAL CYTOTOXIN: DAMAGE TO THE RESPIRATORY EPITHELIUM

Few pathogens produce as many biologically diverse toxins as *B. pertussis*, and TCT is the most recently discovered component of this arsenal. Although a structurally simple molecule, its target cell specificity is so narrow that it had completely escaped detection until assayed with appropriate model systems, cultured respiratory epithelial cells. These and other models should now make it possible to evaluate the precise mechanism by which TCT selectively destroys ciliated cells, a central pathological step in the pertussis syndrome.

Goldman, W. E.

Microbiology pp. 65-69, 1986.

Other support: From the Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis.

IMPROVED ISOLATION OF RAT LUNG ALVEOLAR TYPE II CELLS: MORE REPRESENTATIVE RECOVERY AND RETENTION OF CELL POLARITY

We have developed an important procedure for rat lung alveolar Type II (ATII) cells which yields a more representative sample of ATII cells with respect to their densities. This procedure includes an initial selection on a density gradient of approximately the complete density range of rat lung ATII cells. Subsequently, to exclude contaminating macrophages and lymphocytes from this fraction, the authors have exploited the fact that the contaminating cells have leukocyte common antigen (LC) on their surfaces, whereas the ATII cells do not. Our procedure yields 2×10^7 ATII cells per rat in a fraction which is 90% pure; the cells are immediately available for biochemical or pharmacologic analysis and represent a 90–95% recovery of the ATII cells loaded onto the density gradient. The cells retain their *in vivo* morphologic characteristics, including their polarity.

Weller, N. K. and *Karnovsky, M. J.*

American Journal of Pathology 122:92-100, 1986.

From the Department of Pathology, Harvard Medical School, Boston.

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

ISOLATION OF PULMONARY ALVEOLAR TYPE I CELLS FROM ADULT RATS.

The authors have developed a procedure for the isolation of alveolar Type I (ATI) cells from adult rat lung. After an initial selective enzymatic digestion of the lungs by lavage with 0.2% collagenase, 0.05% trypsin, 0.008% elastase, and 0.005% DNAse Type I, the cells which are released are separated by density gradient centrifugation, and a fraction which includes all ATI cells (density, 1.0177-1.041) is harvested. Contaminating leukocytes are excluded by specific surface adsorption, exploiting the fact that these cells have leukocyte common antigen on their surfaces, whereas ATI cells do not. Similarly, contaminating alveolar Type II (ATII) cells are removed by specific surface adsorption with the use of the lectin *Maclura pomifera* agglutinin, which binds to freshly isolated ATII cells and not to ATI cells. Our procedure yields 5×10^5 ATI cells per rat in a fraction that is at least 85-88% pure, the cells are immediately available for biochemical or pharmacologic analysis and represent a 98% recovery of the ATI cells loaded onto the density gradient. The ATI cells retain their essential *in vivo* morphologic characteristics, including their polarity.

Weller, N. K., and *Karnovsky, M. J.*

American Journal of Pathology **124** 448-456, 1986.

From the Department of Pathology, Harvard Medical School, Boston.

HUMAN ALVEOLAR LINING MATERIAL AND ANTIBACTERIAL DEFENSES.

To investigate the possible antibacterial properties of human alveolar lining material (ALM), we obtained ALM and pulmonary alveolar macrophages (PAM) by bronchoalveolar lavage of healthy nonsmokers. Alveolar lining material was isolated by centrifugation or micropore filtration; electron microscopy revealed lamellar bodies, and lipid analysis showed that 98% of the lipid fraction was phospholipid. No free fatty acids were detected. *Streptococcus pneumoniae* and nontypable *Haemophilus influenzae* (NTHI) died spontaneously in PBS at a mean rate of log 0.75 and 0.95 in 90 min, respectively; the addition of ALM appeared to exert a slight protective effect, and at higher concentrations supported replication of NTHI. There was no difference in the uptake of the bacteria by PAM when ALM was present. Phagocytosed NTHI were killed rapidly and completely within 60 min by PAM with or without ALM. A greater proportion of *S. aureus* were killed by PAM alone than in the presence of ALM. Alveolar lining material from healthy humans thus appears to have no demonstrable effect on host defense against these bacteria. The differences between our results and those of earlier studies using ALM from rats may relate to interspecies differences in the composition of ALM.

Jonsson, S., Musher, D. M., Goree, A., and *Lawrence, E. C.*

American Review of Respiratory Disease **133** 1136-1140, 1986.

Other support: Veterans Administration and the National Institutes of Health.

From the Medical Service (Infectious and Pulmonary Disease Section), Veterans Administration Medical Center, and the Departments of Medicine, Microbiology, and Immunology, Baylor College of Medicine, Houston.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

EVIDENCE AGAINST LEUKOTRIENE-MEDIATION OF PROPRANOLOL-INDUCED AIRWAY HYPERREACTIVITY TO ACETYLCHOLINE

In unanesthetized guinea pigs, propranolol treatment (0.1 mg kg^{-1} i.v.) substantially increased reactivity to intravenous acetylcholine infusion or aerosolized histamine to a comparable degree. Neither BW755c (5 mg kg^{-1} i.v.), FPL 55712 (1 mg kg^{-1} i.v.), nor piriprost (5 mg kg^{-1} i.v.) pretreatment influenced propranolol's effect on muscarinic reactivity although BW755c abolished histaminic hyperreactivity. This suggests that propranolol-induced muscarinic hyperreactivity in the guinea pig is not mediated by leukotrienes whereas histaminic hyperreactivity may be.

Macias, C.

Journal of Pharmacy and Pharmacology **38**:550–552, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

LEUKOTRIENE B$_4$ POTENTIATES AIRWAY RESPONSIVENESS *IN VIVO* AND *IN VITRO*

We studied the effects of leukotriene B$_4$ (LTB$_4$) on guinea pig airway muscle responsiveness *in vivo* and *in vitro*. Responsiveness *in vivo* was assessed by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion in 5 unanesthetized, spontaneously breathing guinea pigs. We found that aerosolized LTB$_4$, in a concentration that itself had no effect on baseline SRaw, caused a substantial increase in bronchial reactivity to i.v. ACh within 8 minutes of its administration. Responsiveness *in vitro* was assessed by measuring isometric contraction of the guinea pig trachealis upon stimulation by either chemical or electrical field stimuli. These studies *in vitro* showed that a concentration of LTB$_4$ that itself did not cause contraction of pretentated airway muscle contraction to ACh and KCl, but not to norepinephrine. This effect of LTB$_4$ was substantially reduced by indomethacin. Our data suggests that an action of LTB$_4$, itself ineffective in constricting the airway *in vivo* or *in vitro*, may directly potentiate the responsiveness of airway smooth muscle to other bronchoconstrictors.

Dethpe, J. E. and *Macias, C. G.*

Prostaglandins **31**(5):898–908, May 1986.

Other support: National Heart, Lung and Blood Institute and the American Lung Association.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

8-BROMOCYCLIC GMP ABOLISHES 5-HT-INDUCED SLOW ACTION POTENTIALS IN CANINE TRACHEALIS MUSCLE

Using intracellular microelectrodes, we investigated whether 8-bromoguanosine

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

3',5'-cyclic monophosphate (cGMP) influenced the electromechanical effects of tetraethylammonium ... TEA on canine tracheal smooth muscle. We found that 20 mM TEA depolarized airway smooth muscle cells from −58 ± 3 mV (mean ± S.D.) to −44 ± 2 mV and caused spontaneous action potentials (APs) to develop which were 31 ± 2 mV in amplitude. These APs, and the phasic contractions electrically coupled to them, were totally abolished in buffer containing 0.1 mM cGMP. Our findings suggest that cGMP markedly affects the channels mediating TEA-induced APs in airway smooth muscle.

Richards, I. S., *Mathias*, C., Ousterhout, J. M., and Sperelakis, N.

European Journal of Pharmacology **128**:299–302, 1986

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association

From the Departments of Medicine, Physiology, and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH

EFFECTS OF LEUKOTRIENE D_4 AND ANTAGONISTS OF ARACHIDONIC ACID METABOLISM AND CALCIUM ENTRY ON GUINEA PIG TRACHEAL MUSCLE RESPONSIVENESS

We studied the effects of antagonists of arachidonic acid metabolism and calcium entry on the responsiveness of airway smooth muscle to acetylcholine (ACh), potassium and norepinephrine. Responsiveness in vitro was assessed by measuring isometric contraction of guinea pig tracheal rings upon chemical stimulation. We found that indomethacin potentiated the response of airway muscle to ACh and KCl, but not to norepinephrine. The indomethacin-induced potentiation observed was inhibited by both BW 755C and nitrendipine. As occurred with indomethacin pretreatment, we also found that an equicontractile concentration of leukotriene D_4 (LTD_4) potentiated the responsiveness of tracheal muscle to both ACh and KCl. Our data suggest that indomethacin or LTD_4 potentiation of guinea pig airway muscle responsiveness to certain bronchoconstrictors may be mediated, at least in part, by enhanced extracellular Ca^{++} entry.

Thorpe, J. E., *Mathias*, C. G.

Prostaglandins, Leukotrienes and Medicine **24**:269–275, 1986

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH

INDOMETHACIN INCREASES BRONCHIAL REACTIVITY AFTER EXPOSURE TO SUBTHRESHOLD OZONE LEVELS

We investigated the effects of indomethacin on bronchial reactivity after ozone exposure. Guinea pigs (two groups of six) were treated with indomethacin (30 mg/kg IP) and studied 1 hr later and 24 hr after a 2-hr exposure to either 1.5 or 3.0 ppm ozone. These

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

animals were compared to similarly exposed groups that were untreated. Reactivity was determined by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion. Prior to ozone exposure, indomethacin had no effect on either SRaw or muscarinic reactivity. In all untreated guinea pigs (n = 12) exposed to 1.5 ppm ozone, there was no significant change in SRaw or muscarinic reactivity. In contrast, all treated animals exposed to 1.5 ppm showed a substantial increase in reactivity. Those treated animals exposed to 3.0 ppm showed significant elevations in SRaw, making interpretations of changes in their reactivity difficult. We conclude that indomethacin treatment increases bronchial reactivity in guinea pigs exposed to subthreshold ozone levels.

Murlas, C. et al.

Prostaglandins Leukotrienes and Medicine 21:259-268, 1986.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

K^+-INDUCED ALTERATIONS IN AIRWAY MUSCLE RESPONSIVENESS TO ELECTRICAL FIELD STIMULATION

We investigated possible pre- and postsynaptic effects of K^+-induced depolarization on ferret tracheal smooth muscle (TSM) responsiveness to cholinergic stimulation. To assess electromechanical activity, cell membrane potential (E_m) and tension (T_e) were simultaneously recorded in buffer containing 6, 12, 18, or 24 mM K^+ before and after electrical field stimulation (EFS) or exogenous acetylcholine (ACh). In 6 mM K^+, E_m was -58.1 ± 1.0 mV (mean $\pm$ SE). In 12 mM K^+, E_m was depolarized to -52.3 ± 0.9 mV, basal T_e did not change, and both excitatory junctional potentials and contractile responses to EFS at short stimulus duration were larger than in 6 mM K^+. No such potentiation occurred at a higher K^+, although resting E_m and T_e increased progressively above 12 mM K^+. The sensitivity of ferret TSM to exogenous ACh appeared unaffected by K^+. To determine whether the hyperresponsiveness in 12 mM K^+ was due, in part, to augmented ACh release from intramural airway nerves, experiments were done using TSM preparations incubated with [^{3}H]choline to measure [^{3}H]ACh release at rest and during EFS. Although resting [^{3}H]ACh release increased progressively in higher K^+, release evoked by EFS was maximal in 12 mM K^+ and declined in higher concentrations. We conclude that small elevations in the extracellular K^+ concentration augment responsiveness of the airways, by increasing the release of ACh both at rest and during EFS from intramural cholinergic nerve terminals. Larger increases in K^+ appear to be inhibitory, possibly due to voltage-dependent effects that occur both pre- and postsynaptically.

Mathias, C. et al.

Journal of Applied Physiology 61(1):61-67, 1986.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

EFFECTS OF MUCOSAL REMOVAL ON GUINEA PIG AIRWAY SMOOTH MUSCLE RESPONSIVENESS

1. The contractile response to histamine, acetylcholine (ACh), KCl or electrical field stimulation (EFS) was examined in paired tracheal rings, one of each being denuded by mucosal rubbing, which were mounted in muscle chambers and bathed with a continuously aerated physiological salt solution at 37° C.

2. Removal of the respiratory mucosa increased the sensitivity of airway muscle to ACh, histamine and EFS, but not to KCl. The hypersensitivity of denuded rings to histamine and EFS was greater than to ACh. Atropine reduced the histamine hypersensitivity observed.

3. Pretreating tissue preparations with indomethacin augmented their responsiveness to EFS, histamine and ACh.

4. Indomethacin augmentation of histamine- and EFS-induced responses was greater in preparations without epithelium.

5. We conclude that the airway mucosa may be associated with a factor that reduces airway smooth muscle responsiveness to stimulation.

Marlies C

Clinical Science (70.5?)[5?5], 1986

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association

From the Department of Medicine, Physiology and Environmental Health, University of Cincinnati, School of Medicine, Cincinnati, OH

O3-INDUCED CHANGE IN BRONCHIAL REACTIVITY TO METHACHOLINE AND AIRWAY INFLAMMATION IN HUMANS

The increase in airway responsiveness induced by O3 exposure in dogs is associated with airway epithelial inflammation, as evidenced by an increase in the number of neutrophils, epithelial nuclei, lymphocytes, basophils in epithelial biopsies and in bronchoalveolar lavage fluid. We investigated in 10 healthy human subjects whether O3-induced hyperresponsiveness was similarly associated with airway inflammation by examining changes in the types of cells recovered in bronchoalveolar lavage fluid obtained after exposure to air or to O3 (0.4 or 0.6 ppm). We also measured the concentrations of cyclooxygenase and lipoxygenase metabolites of arachidonic acid in lavage fluid. We measured airway responsiveness to inhaled methacholine aerosol before and after each exposure and performed bronchoalveolar lavage 3 h later. We found more neutrophils in the lavage fluid from O3-exposed subjects, especially in those in whom O3 exposure produced an increase in airway responsiveness. We also found significant increases in the concentrations of prostaglandins E_2, I_2, and thromboxane B_2 in lavage fluid from O3-exposed subjects. These results show that in human subjects O3-induced hyperresponsiveness to methacholine is associated with an influx of neutrophils into the airways and with, but not the levels of, airway cyclooxygenase metabolites of arachidonic acid.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

Seltzer, J., Bigby, B. G., Stulbarg, M., and *Nadel, J. A.*

Journal of Applied Physiology **60**(4):1321-1326, 1986.

Other support: National Heart, Lung, and Blood Institute, California Air Resources Board, Fisons Corporation, Vick Divisions Research, and the National Cystic Fibrosis Foundation.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

ERYTHROCYTES FROM CIGARETTE SMOKERS CONTAIN MORE GLUTATHIONE AND CATALASE AND PROTECT ENDOTHELIAL CELLS FROM HYDROGEN PEROXIDE BETTER THAN DO ERYTHROCYTES FROM NONSMOKERS

Recent observations regarding the ability of intracellular erythrocyte (RBC) antioxidants to decrease O_2^- metabolite-mediated injury to lung tissues have prompted interest in the RBC antioxidants of patients with lung disease. We found that RBC from 14 healthy, age- and gender-matched cigarette smokers contained more ($p < 0.05$) glutathione (6.3 ± 0.4 μM g Hgb) versus 5.0 ± 0.3 μM g Hgb) and catalase (249,533 ± 8,307 units g Hgb versus 222,617 ± 7,180 units g Hgb) than did RBC from nonsmokers. In contrast, RBC from cigarette smokers and nonsmokers contained the same activities of glutathione peroxidase (21.4 ± 1.2 units g Hgb versus 20.4 ± 5.5 units g Hgb). RBC from cigarette smokers also protected bovine pulmonary artery endothelial cells in culture from hydrogen peroxide (H_2O_2) better ($p < 0.05$) than did RBC from nonsmokers (52.1 ± 6.1% protection versus 31.9 ± 5.7% protection). The results suggest that alterations in RBC antioxidants may reflect exposure and/or affect susceptibility to oxidant-induced injury.

Toth, K. M., Berger, E. M., Beehler, C. J., and *Repine, J. E.*

American Review of Respiratory Disease **134**(2):281-284, 1986.

Other support: National Institutes of Health, American Lung Association, Procter & Gamble, and Tambrands, Inc.

From the Departments of Medicine and Pediatrics and the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

PROCESSING OF ANGIOTENSIN AND OTHER PEPTIDES BY THE LUNGS

Understanding the interactions of soluble or circulating components of the renin-angiotensin system and the kallikrein-kinin system with cellular components is important because both systems function, at least in some respects, as local hormones. However, even their systemic actions may presume critically important reactions with cellular components that are not uniformly distributed within organs or within particular cell types of a given organ or tissue. One unique feature of the pulmonary circulation is the remarkable selectivity of its metabolism of these peptides. Over the past several years, aspects of the metabolism of kinins and angiotensin I by pulmonary endothelium have been defined. The focus of this author has been on pulmonary endothelial cells which selectively process a series of vasoactive substances. Studies have shown that endothelial cells of the lung can be considered a very complex solid

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

phase reactor capable of extremely fast reactions of high selectivity, perhaps even specificity. This chapter details the known characteristics, functions and metabolism of the bradykinin-angiotensin family of peptides in the pulmonary circulation and includes a discussion of recent advances in the study of relevant pulmonary enzymes. The utility of cultured pulmonary endothelial cells as a model system is also evaluated and certain clinical aspects of the kallikrein-kinin and renin-angiotensin systems are reviewed and discussed.

Ryan, U. S.

In: Fishman, A. P. and Fisher, A. B., eds. *Handbook of Physiology. The Respiratory System II*. American Physiological Society, 1985, pp. 351-364.

Other support. National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

METABOLIC ACTIVITY OF PULMONARY ENDOTHELIUM MODULATIONS OF STRUCTURE AND FUNCTION

Endothelial cells play a number of active roles. They act as a barrier with limited permeability, they process circulating hormones and other bioactive materials with great efficiency and selectivity, they share the responsibility for hemostatic reactions with cells and components of blood, but under normal conditions interact very little with blood cells or blood-borne clotting factors. The enzymes, enzyme inhibitors, receptors, and transport proteins responsible for the outcome of interactions of endothelial cells with circulating substances are in many cases known. Some of the active substances released by endothelial cells have also been documented, but undoubtedly many remain to be discovered. The active antithrombogeneity of endothelium is probably due in part to release of prostacyclin, but even when prostacyclin is inhibited endothelium does not become thrombogenic. In fact, only when endothelial cells in vitro are transformed do they become thrombogenic. Under these circumstances the polarity of the endothelium may be lost and the distinction between the glycocalyx on the luminal surface and the basement membrane materials on the abluminal surface may become confused. The full range of properties and functions of the normal glycocalyx are not known, but it is clear that endothelium can play an active role in the events leading to microvascular occlusion and damage. The endothelial surface over which blood normally flows unimpeded can become a focus for procoagulant and complement-linked reactions, and such changes may be accompanied by a disarray of the glycocalyx. It is tempting to speculate that the glycocalyx is important in the recognition processes between endothelial cells and blood elements and that alterations in hemostatic and immunologic potentials are accompanied by alterations in the endothelial glycocalyx.

Ryan, U. S.

Annual Review of Physiology **48**:263-277, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

RECEPTOR-MEDIATED BINDING OF C1q ON PULMONARY ENDOTHELIAL CELLS

Normal undamaged pulmonary endothelial cells appear to be immunologically privileged in that they do not express receptors for the Fc portion of IgG nor for C3b. However, these receptors become unmasked on endothelial cells injured by viral infection or exposure to white cell lysates. We now present evidence to indicate that C1q binds to specific receptors on the surface of normal healthy endothelial cells. The binding is dose-dependent, reversible and saturable. Furthermore our data show that binding of C1q to endothelial cells is via the collagenous portion of the molecule not via the globular head regions. Thus binding of C1q to endothelium would have the effect of exposing Fc receptors that could then bind to IgG of circulating immune complexes. That Fc receptors are in fact exposed is shown by rosette formation with antibody sensitized erythrocytes. With 2C1r-2C1s-associated C1q, no binding occurred using gel fixation and transfer assays. Our results indicate that C1q binding to endothelium provides a means for localizing immune complexes on pulmonary vessels and may be important in the initiation and progression of the inflammatory response.

Zhang, S. C., Schultz, D. R. and *Ryan, U. S.*

Tissue and Cell **18** (1) 13–18, 1986.

Other support: U. S. Public Health Service.

From the University of Miami School of Medicine, Department of Medicine, Miami, FL.

REACTIONS AT THE PULMONARY ENDOTHELIAL CELL SURFACE

Since 1967, it has become evident that a wide variety of biochemical reactions occur at the surface of vascular endothelium. Many of these reactions appear to occur continuously (e.g., metabolic processing of vasoactive substances) while others occur episodically (e.g., expression of procoagulant activities and interactions with complement). Certain of both types of reactions are now understood in fair detail and in terms of structure-function correlations. The text that follows focuses 1) on continuous interactions of endothelial surface peptidase enzymes with blood-borne oligopeptide hormones, and 2) on induction of procoagulant activities and complement and immune component receptors consequent to endothelial injury. The episodic activities appear related in part to disarray of the normally highly organized glycocalyx. Of the continuous activities, the most clearly understood are the interactions of endothelium with two oligopeptides, the hormone bradykinin and the prohormone angiotensin I. Angiotensin I is converted into the vasopressor hormone angiotensin II, and bradykinin, a vasodepressor hormone, is inactivated by angiotensin converting enzyme. The latter enzyme is disposed on the endothelial surface such that it has virtually free access to its blood-borne substrates. As a consequence, endothelium can be said to regulate the hormonal composition of blood moving downstream. In the case of pulmonary endothelium, downstream blood is systemic arterial blood.

Ryan, U. S. and Ryan, J. W.

Current Methods of Transport of Solutes from Blood to Tissue, Longman, London, 1985, pp. 223–236.

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

Other supp[illegible] National Heart, Lung, and Blood Institute of the National Institutes of Health

From the [illegible] of Medicine, University of Miami School of Medicine, Miami, FL

THE ENDOTHELIAL SURFACE AND RESPONSES TO INJURY

Pulmonary microvascular endothelial cells are known to play an active role in the events that follow vascular damage in the inflammatory response. The endothelial surface is normally physically privileged and very actively antithrombogenic, can respond to certain stimuli generally injurious by becoming strongly procoagulant and by expressing C3 and C3b receptors. Like macrophages, activated endothelial cells can provide a procoagulant source and substratum for combined hemostatic and complement-linked [illegible]. Such transformations of endothelial functions may involve alterations in the [illegible] of glycocalyx and, in addition, may be important for the entrapment and [illegible] of phagocytosed particulates. What is clear is that it is not simply absence of endothelium that has a bearing on the outcome of inflammatory stimuli but the [illegible] structural and functional responses of the endothelial surface to injury, resulting in altered expression of hemostatic and immunologic potential, may have an important bearing on the role of the endothelium in the regulation of microvascular permeability.

Rese[illegible]

Federation Proceedings 45 (suppl 10): 108, 1986

Other support: National Institutes of Health

From the Department of Medicine, University of Miami School of Medicine, Miami, FL

PULMONARY ENDOTHELIAL CELL KILLING BY HUMAN NEUTROPHILS: POSSIBLE INVOLVEMENT OF HYDROXYL RADICAL

Human blood neutrophils stimulated by a variety of agents were shown to have cytotoxic effects on bovine pulmonary artery endothelial cells. Effective agonists included immune complexes, opsonized zymosan, and 12-*O*-tetradecanoyl phorbol acetate. Unstimulated human neutrophils and neutrophils stimulated with *N*-formyl methionyl leucyl phenylalanine or with platelet activating factor failed to induce significant killing even though secretory release of lysosomal enzymes occurred. In comparing the [illegible] of endothelial cell killing showed a better correlation with the production of H_2O_2 than with the generation of O_2^-. Endothelial cell killing by stimulated human neutrophils was inhibited by catalase but not by soybean trypsin inhibitor or superoxide dismutase. Killing was also inhibited by two scavengers of hydroxyl radical, dimethylsulfoxide and D-mannitol, and by deferoxamine mesylate, an iron chelator. Iron-saturated deferoxamine mesylate was significantly less effective in protecting the endothelial cells against killing. Agents that were protective against endothelial cell killing did not interfere with the generation of O_2^- by stimulated neutrophils. These results suggest that leukocyte-induced endothelial cell killing is mediated by [illegible] by some but not all agonists from neutrophils and that the killing mechanism may be directly due to hydroxyl radical production.

National [illegible] Society [illegible] of Clinical and Experimental [illegible]

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

Journal of Pharmacology and Experimental Therapeutics, 253:(1990) [illegible]

Other support: University of Michigan and the University of Miami.

[illegible] Department of Medicine, University of Miami School of Medicine, Miami, FL

DIRECT EFFECTS OF *E. COLI* ENDOTOXIN ON STRUCTURE AND PERMEABILITY OF PULMONARY ENDOTHELIAL MONOLAYERS AND THE ENDOTHELIAL LAYER OF INTIMAL EXPLANTS

The direct structural, metabolic, and physiologic effects of *Escherichia coli* endotoxin on bovine pulmonary endothelial monolayers and on the intact endothelial layer of bovine pulmonary artery intimal explants were examined. Endothelial monolayers exposed to *E. coli* endotoxin (0.001, 0.01, 0.1, 1.0, and 10 μg/ml) for 24 hours in the absence of bovine fetal calf serum (FCS) showed a dose-dependent response, as demonstrated by number of pyknotic cells and lactate dehydrogenase release that was enhanced by addition of FCS. Prostacyclin production was increased only in the presence of FCS. Endotoxin also caused an increase in permeability. Endothelial cells [illegible] seeded on filters placed in chemotaxis chambers with radioactive tracers in the upper well showed a significant 25% increase in tracer equilibration (counts in lower well/counts in upper well) at 24 hours' incubation with endotoxin (0.89 ± 0.05 mean ± SE) vs. no endotoxin (0.69 ± 0.05) and a 40% increase in equilibration of ^{125}I-albumin at three hours (3 hours' endotoxin = 0.40 ± [illegible] vs. no endotoxin = 0.27 ± 0.02). An increase in hydraulic conductance was also seen at 48 hours. Electron microscopy of the endothelial layer of intimal explants showed disruption of the intercellular junctions and cellular changes representing contraction, increased prominence of cytoplasmic filaments, nuclear crenations, and cytoplasmic projections at 30 and 60 minutes. From 2 hours evidence of cell death was found. These findings show that endotoxin causes structural and metabolic changes in pulmonary endothelial cells and an increase in permeability of the endothelial layer. This injury occurs in the absence of FCS but is enhanced by its addition.

M. [illegible] E. Orr, Rose [illegible] U. S. and Brigham, K. L.

American Review of Respiratory Disease, 122:143–151, 1980.

Other support: National Heart, Lung and Blood Institute and the Knox Foundation.

[illegible] Medicine, University of Miami School of Medicine, Miami, FL

GLUCOCORTICOID MODULATION OF PROSTACYCLIN PRODUCTION IN CULTURED BOVINE PULMONARY ENDOTHELIAL CELLS

The present study has examined the ability of glucocorticoids to modify the production of prostacyclin by endothelial cells derived from bovine pulmonary artery. Binding studies with ^{3}H-dexamethasone indicated that these cells possessed high-affinity binding sites for glucocorticoids (K_d approximately 4 nM). The cells released [illegible] measured serologically as its hydrolysis product 6-keto-prostaglandin F. The release was strongly stimulated by a 5-min incubation with 500 nM bradykinin.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

2 μM calcium ionophore A23187 and 0.5 μM arachidonic acid. Indomethacin (1 to 2½ μM) suppressed prostacyclin release in response to bradykinin and ionophore in a dose-dependent manner. The suppressive effects required 20 to 30 minutes of exposure. Maximal suppression of bradykinin-induced prostacyclin release was approximately 65% and that of ionophore-induced release was approximately 75%. Equivalent suppression was not observed. Hydrocortisone, at 2½ μM, inhibited bradykinin-induced release of prostacyclin by approximately 45%, but had no effect on ionophore-induced release. Neither glucocorticoid inhibited prostacyclin release in response to arachidonic acid. Trifluoperazine and combined media from cells treated with 2½ μM aspirin or 2½ μM Triton X-100 were also used. Indomethacin, hydrocortisone, trifluoperazine, and aspirin in some failed to modify prostacyclin release when added to the perfusion medium. We conclude that diverse mechanisms are operative in the production of prostacyclin by binding site occupancy, inhibits agonist-induced release of prostacyclin from bovine pulmonary endothelial cells. Inhibition appears to occur at the level of intracellular arachidonic acid release. However, we have been unable to obtain direct evidence for the production of a macromolecular phospholipase A2 by endothelial cells in response to these low concentrations of stimuli.

Critchley, D. J., Rose, G. S., and Ryan, J. W.

The Journal of Pharmacology and Experimental Therapeutics 233 (2): 651–655, 1985.

Other support: U. S. Public Health Service and the Danielle Phipps Memorial Cardiology Trust.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL

SINGLE APICAL MEMBRANE ANION CHANNELS IN PRIMARY CULTURES OF CANINE RENAL PROXIMAL TUBULE CELLS

To investigate the cellular mechanisms responsible for the apical membrane Cl conductance in a tight epithelium, we used the patch-clamp technique to study single-ion channels in primary cultures of canine tracheal epithelium. The cells contained an anion channel that had a single-channel conductance of approximately 2 pS at negative voltages (cytosol vertical, 145 mM; bath Cl, 5 mM). In symmetrical Cl solutions, the excised single-channel current-voltage relation was linear with conductance of 2 pS. The current-voltage relation was rectified by the presence of 2-carboxyarylaminobenzoic acid (CAB) at concentrations similar to those required to block the cAMP-activated Cl conductance. The channel was activated by dibutyryl cAMP in cell-attached patches from intact cells, isolated cells, and excised patches. The apical membrane of confluent cells was usually inactive. When activated, the channel was also activated by an increase of cytosolic Ca from the bath. Other anions were tested for permeation through the pore. Cytosolic ATP, excised from the cell, did not block the channel. Cytosolic pH, however, modulated the channel activity by altering the open probability. The results suggest that this channel may be responsible for the apical Cl conductance in tracheal epithelium.

Welsh, M. J.

Pflügers Archiv: European Journal of Physiology 405 (Suppl. 1): S139–S146, 1985.

Other support: N[illegible]

From the Department of Physiology, Iowa Regional Primate Research Center, University of Iowa, Iowa City, IA

Source: https://www.industrydocuments.ucsf.edu/docs/hqxk0000

CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CANINE AIRWAY SMOOTH MUSCLE

1. Airway smooth muscle cells from canine trachealis muscle were dispersed by treatment with collagenase and elastase. Cells were identified as smooth muscle by their binding of anti-smooth muscle myosin monoclonal antibodies and by their contraction in response to acetylcholine. 2. The patch-clamp technique was used to study single channel currents in cell-attached and isolated patches of membrane. The most common single channel current had a conductance of 266 ± 12 pS (mean ± SD, $n = 70$) in symmetrical 135 mM K solutions. 3. The reversal potential of the channel was unaltered by large chemical gradients for Cl, Na and Ca and was determined exclusively by the chemical K gradient. Thus, the channel is highly selective for K. 4. In both cell-attached and isolated patches of membrane, depolarization increased the frequency of channel opening and the duration of the open state. 5. In isolated patches of membrane, increasing [Ca] on the cytoplasmic side of the membrane from 10^{-7} to 10^{-6} M increased both the frequency of channel opening and the duration of the open state. 6. Tetraethylammonium, tetramethylammonium, or Cs (10 mM) on the cytoplasmic side of the membrane caused a voltage-dependent decrease in conductance of the open channel while having no obvious effect on channel kinetics. These blocks were completely reversible. Ba (10 mM) on the cytoplasmic side of the membrane slightly decreased inward currents and completely blocked outward currents through the channel. 7. External Ba (10 mM) caused a voltage-dependent decrease in inward current. External tetraethylammonium (TEA) (5 mM) completely blocked single channel currents.

McCann, J. D., and Welsh, M. J.

Journal of Physiology **372**:113-127, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

ADRENERGIC REGULATION OF ION TRANSPORT BY PRIMARY CULTURES OF CANINE TRACHEAL EPITHELIUM: CELLULAR ELECTROPHYSIOLOGY

We examined the effect of adrenergic agents on the [illegible] of primary cultures of canine tracheal epithelium. Both isoproterenol and epinephrine stimulated Cl secretion, as evidenced by an increase in transepithelial voltage and a fall in transepithelial resistance. Moreover, both agents appear to increase the conductance of apical and basolateral membranes. However, the pattern of response was different. Isoproterenol initially depolarized apical voltage (Ψ_a) and decreased the fractional resistance of the apical membrane (f_a). These changes are consistent with an initial increase in apical Cl conductance. In contrast, epinephrine acutely hyperpolarized Ψ_a and increased f_a, changes consistent with an initial increase in basolateral K conductance. Following the acute effect of epinephrine, Ψ_a depolarized and f_a decreased to values not significantly different from those observed with isoproterenol. The acute increase in basolateral K conductance produced by epinephrine appeared to result from stimulation of α-adrenergic receptors because it was reproduced by addition of the α agonist phenylephrine, and blocked by the α antagonist phentolamine. The ability of prazosin

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but not yohimbine, to block the acute epinephrine induced increase in K permeability indicates the presence of α2 adrenergic receptors. The acute α adrenergic-induced increase in basolateral K conductance may be mediated by an increase in cell Ca because the response was mimicked by addition of the Ca ionophore A23187. In contrast, the response to isoproterenol was similar to that observed with addition of 8-bromo-cAMP and theophylline. These results indicate that both β and α adrenergic agents mediate the ion transport processes in canine tracheal epithelium. β adrenergic agents have their primary effect on the apical Cl conductance, probably via an increase in cAMP. α adrenergic agents exert their primary effect on the basolateral K conductance, possibly via an increase in cell Ca.

Welsh, M. J.

The Journal of Membrane Biology **91**:121–128, 1986.

Other support: National Institutes of Health, and the American Heart Association.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

MUCOSAL ADENOSINE STIMULATES CHLORIDE SECRETION IN CANINE TRACHEAL EPITHELIUM

Adenosine is a local regulator of a variety of physiological functions in many tissues and has been observed to stimulate secretion in several Cl secreting epithelia. In canine tracheal epithelium we found that adenosine stimulates Cl secretion from both the mucosal and submucosal surfaces. Addition of adenosine, or its analogue 2-chloroadenosine, to the mucosal surface potently stimulated Cl secretion with no effect on the rate of Na absorption. Stimulation resulted from an interaction of adenosine with adenosine receptors, because it was blocked by the adenosine receptor blocker, 8-phenyltheophylline. The adenosine receptor was a stimulatory receptor as judged by the rank order potency of adenosine and its analogues and by the increase in cellular adenosine 3',5'-cyclic monophosphate levels produced by 2-chloroadenosine. Adenosine also stimulated Cl secretion when it was added to the submucosal surface, although the maximal increase in secretion was less and it was much less potent. Part, but not all, of the lower potency of submucosal adenosine resulted from submucosal uptake and metabolism of the drug. The observation that mucosal 8-phenyltheophylline blocked the effect of submucosal 2-chloroadenosine, whereas submucosal 8-phenyltheophylline did not prevent a response to mucosal or submucosal 2-chloroadenosine, suggests that adenosine receptors are located on the mucosal surface. Thus, submucosal adenosine may stimulate secretion by crossing the epithelium and interacting with receptors located on the mucosal surface. Because adenosine can be released from mast cells located in the airway lumen in response to inhaled materials and because adenosine stimulated secretion from the mucosal surface, it may be a unique positive control like epithelium or a regional level.

Bhatt, A. D., Clancy, G. and Welsh, M. J.

American Journal of Physiology, **251**:C96–C103, 1986.

Other support: National Institutes of Health, and Research Institute.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

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III. *Heart and Circulation*

COMPARTMENTATION AND FUNCTIONAL MECHANISMS IN MYOCARDIAL FAILURE AND MYOCARDIAL INFARCTION

Changes in compartmentation and specific mechanisms in acute myocardial failure due to global ischemia and in regional myocardial ischemia in dog hearts are described. Ischemic failure was produced by periodic arrest of flow to supported heart preparations perfused with a fluorocarbon (FC-43). Sarcolemmal vesicles (SL) prepared from ischemic failing heart preparations exhibited diminished Ca^{++} binding and phosphorylation. TA-064, a beta-1 agonist, partially abolished the reduction in Ca^{++} binding and phosphorylation of SL vesicles. The addition of cyclic-AMP (cAMP) and of protein kinase (PK) increased phosphorylation of SL vesicles obtained from nonfailing heart preparations. Combination of cAMP and of PK had the greatest effect. In contrast to myocardial failure, myocardial infarction is known to produce a large variety of specific disturbances in intermediary cardiac metabolism. Apparently in ischemic failing heart preparations, Ca^{++} binding and phosphorylation of SL are decreased. The results with TA-064 and isoproterenol suggest that phosphorylation of SL may play a role in the positive inotropic effect of beta-1 agonists.

Key Words: [illegible]

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From the Huntington Medical Research Institutes, Pasadena, CA.

EFFECT OF 8-BROMO-CYCLIC GUANOSINE MONOPHOSPHATE (cGMP) ON CORONARY ARTERY CONSTRUCTION IN ISOLATED RABBIT HEARTS

The vasodilator 8-bromo-guanosine 3',5'-monophosphate (8-bromo-cGMP) effectively counteracts vasopressin-induced coronary artery constriction in a supported perfused working rabbit heart. In this preparation, the coronary arteries remain in contact with the beating heart. The obtuse marginal artery and portions of the left anterior descending coronary artery were deprived of endothelium. Perfusion was carried out with Krebs-Henseleit solution, oxygenated with disposable intan oxygenator. The internal diameter of large coronary arteries was determined by roller arteriography (injection of patent blue dye and [illegible] photography). The effect of vasopressin was tested without the [illegible] of 8-bromo-cGMP on cardiac performance (cardiac [illegible] systolic pressure, left ventricular end-diastolic pressure, maximal [illegible] left ventricular pressure [dP/dt]), mean aortic pressure and large coronary vessel radii. Total coronary vascular resistance was determined in later experiments. In addition, changes in coronary sinus partial pressure of carbon dioxide (P_{CO_2}) and pH were observed. Vasopressin alone caused a significant decline in coronary flow, myocardial oxygen consumption and coronary sinus pH. Cardiac performance

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Saeed, M., Schmidt, J., Metz, M., and Bing, R. J.

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Other support: Hoover Foundation and The Lindberg Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

PHOSPHODIESTERASE INHIBITION IN POSITIVE INOTROPIC THERAPY OF CONGESTIVE HEART FAILURE

Congestive heart failure, which affects approximately two million people in the United States, still carries a poor prognosis. Means of treatment available at the present time require additional options. Recently, a new group of compounds has been clinically and pharmacologically investigated. These agents, the so-called new positive inotropic agents, are not related to the glycosides or the beta-adrenergic stimulants. Common features of these compounds are a combination of positive inotropic effect and vasodilation, a useful combination in the treatment of congestive heart failure. The mechanism of action of these drugs consists of inhibition of myocardial phosphodiesterases with an increase in cyclic 3′,5′-adenosine monophosphate (cAMP). Intracellular [illegible] is increased through a series of reactions and the cardiac performance is improved. Other mechanisms which may play an additional role in the increase of the cardiac contractility are also discussed in this article. Experimental data on the effect of the phosphodiesterase inhibition CI 914 on cardiac performance and vascular beds, as obtained in our laboratory, are included in this report.

Blackburn, A., and Saeed, M. (*Biog. R. J.*)

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Other support: The Margaret W. and Herbert Hoover, Jr. Foundation.

From the Huntington Medical Research Institutes, Pasadena, CA.

MICROVASCULAR DISTRIBUTION OF CORONARY VASCULAR RESISTANCE IN BEATING LEFT VENTRICLE

To determine the distribution of resistance in the coronary vasculature, measurements of microvascular pressure and diameter were obtained with vasomotor tone maintained during coronary dilation produced by papaverine. We studied anesthetized, open-chest cats on fixed-jet ventilation synchronized to the cardiac cycle to eliminate respiratory-induced cardiac motion. The system for measuring microvascular pressure compensated for cardiac motion with stroboscopic illumination of the microvessels and a beat-pulse controlled electromechanical micromanipulator that moved a micropipette in synchrony with the heart. Pressures were measured with the servonull technique and diameters were measured via a video system. Resistance was estimated from the pressure gradient from the aorta to a particular class (size) of coronary [illegible]. During control conditions, with coronary vasomotor tone intact, mid-coronary [illegible] was 36 ± 3 mmHg (mean ± 1 SE) and was increased to 339 ± 52 during

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From the Cardiovascular Center and Department of Internal Medicine, University of Iowa College of Medicine, Iowa City

DNA DAMAGE INDUCED BY NONIONIZING RADIATION IN MOUSE MYOCARDIUM

Gambia, J. W.

Journal of Molecular and Cellular Cardiology 18 (1986) [illegible]

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From the Department of Biochemistry, University of South [illegible] College of Medicine, Melville

PARTICIPATION OF ENDOTHELIAL CELLS IN THE PROTEIN C–PROTEIN S ANTICOAGULANT PATHWAY: THE SYNTHESIS AND RELEASE OF PROTEIN S.

The protein C–protein S anticoagulant pathway is closely linked to the endothelium. In this paper the synthesis and release of the vitamin K–dependent anticoagulant factor protein S is demonstrated. Western blotting after SDS-PAGE of Triton X-114 extracts of bovine aortic endothelial cells grown in serum-free medium demonstrated the presence of protein S. A single major band was observed at [illegible] reacting with protein S purified from plasma absent from cells treated with sodium warfarin. Metabolic labeling of endothelial cells with [^{35}S]methionine confirmed the synthesis of protein S. When a radioimmunoassay was used, endothelium was found to release 150 fmol/10^6 cells per 24 h and contain 44 fmol/10^6 cells of protein S antigen. Protein S released from endothelium was functionally active and could promote activated protein C–mediated factor V$_a$ inactivation on the endothelial cell surface. Warfarin decreased secretion of protein S antigen by >90% and increased intracellular accumulation by almost twofold. Morphologic studies demonstrated that intracellular protein S was in the Golgi complex, concentrated at the *trans* face, rough endoplasmic reticulum, lysosomes, and in vesicles at the periphery. In contrast, protein S was not found in vascular fibroblasts or smooth muscle cells. A pool of intracellular protein S could be released rapidly by the calcium ionophore A23187 (5 μM). This effect was dependent on the presence of calcium in the culture medium and could be blocked by EGTA, which suggests that cytosolic calcium flux may be responsible for protein S release. These results demonstrate that endothelial cells, but not the subendothelial cells of the vessel wall, can synthesize and release protein S, which indicates a new mechanism by which the inner lining of the vessel wall can contribute to the prevention of thrombotic events.

Stern, D., Brett, J., Harris, H., and Nossel, H. L. *Proc. Natl. Acad. Sci. U.S.A.*

The Journal of Cell Biology 102 1971–1978, 1986.

Other support: Young Investigator Award from the Oklahoma affiliate of the American Heart Association, and the National Institutes of Health.

From: Thrombosis Research, Oklahoma Medical Research Foundation, Oklahoma City, and the Department of Pathology, College of Physicians & Surgeons, Columbia Presbyterian Medical Center, New York.

ELUCIDATION OF THE PATHOGENESIS OF THE ATHEROSCLEROTIC PROCESS: LETTING THE GENIE OUT OF THE CULTURE BOTTLE.

The author acknowledges both the past and future importance of molecular and cellular approaches to atherogenesis, but maintains that true understanding of that process must increasingly depend on studies in the intact artery or at least perfused arterial segments. Cell-culture studies, for all their value and power, can only tell us what cells are capable of under specific conditions. For instance, many conclusions regarding LDL uptake, transport and degradation using culture techniques simply were not validated in *in vivo* experiments. Likewise, post secretory modifications of lipoproteins and cell surface antigens will only be fully understood in integrated systems. Examples of other important aspects of the pathogenesis of atherosclerosis that will need *in vivo* studies are the overall dynamics of lipoproteins into the artery wall and the significance of monocyte/macrophage entry into the artery wall. The role of

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[illegible]

Source of Data: [illegible]

[illegible]

Other support: [illegible]

[illegible]

MECHANISMS INVOLVED IN THE UPTAKE AND DEGRADATION OF LOW-DENSITY LIPOPROTEIN BY THE ARTERY WALL *IN VIVO*

The causative linkage between hypercholesterolemia and atherosclerosis is firmly established on laboratory but not clinical evidence. The most convincing evidence from the Lipid Research Clinics intervention trial, which clearly demonstrated that lowering LDL levels in hypercholesterolemic men significantly reduced the risk of death from coronary artery disease. Since the cholesterol in atherosclerotic lesions derives primarily from the cholesterol in circulating lipoproteins, it is reasonable to conclude that hypercholesterolemia is caused by virtue of the discovery that cholesterol enters the artery at a high rate. Thus, although LDL levels can be high, rates of LDL entry into the artery wall are unlikely to be high unless they are driven by cells in the artery wall that are trapping the cholesterol-rich material. The overall balance which normally mediates must normally operate to prevent accumulation of cholesterol and LDL. The endothelial monolayer lining the artery wall is a barrier to the entry of LDL. What we have done is simply to restate the hypothesis of Virchow in slightly modified contemporary form. However, the cause-effect linkage could be quite different. High plasma concentrations of LDL have been [illegible] with varying degrees of certainty implicated as contributing to several other processes that may be involved in atherogenesis, as discussed in detail elsewhere, and there may be still others not yet appreciated. One or more of such factors might be equally relevant or even more relevant to lesion development than the rate of delivery of cholesterol to the tissue. A crucial question is whether the delivery of lipoprotein to and the accumulation of cholesterol in the artery wall is [illegible] the atherogenesis. Certainly cholesterol accumulation is the hallmark of the [illegible] atherosclerotic lesion. Yet in all experimental lesions studied to date [illegible] of causative events was triggered by [illegible] LDL levels were high during lesion development [illegible] if we look at established [illegible] of the lesion, LDL [illegible] is completely reversed [illegible] of possible use in the treatment of this [illegible] LDL [illegible]

Source of Data: [illegible]

[illegible] 454 [illegible]

Other support: [illegible]

[illegible] CA

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LIPOPROTEINS AND ATHEROGENESIS: CURRENT CONCEPTS

Atherosclerosis is undoubtedly a disorder of multiple etiology. Many risk factors are involved in addition to hyperlipoproteinemia—notably cigarette smoking and hypertension. The precise mechanisms by which these other risk factors are linked to the atherogenic process remain to be determined. In the case of lipoproteins, we have made remarkable progress in the past decade toward a well-defined hypothesis for atherogenesis. Already we see the potential for novel modes of intervention to be used to simply modify plasma levels of lipoproteins. As we begin to learn more about the biologic processes within the artery wall that play a role in the atherogenic process, we should develop modes of intervention that will act on these processes. For example, if macrophage secretions are indeed responsible in part for endothelial damage, then drugs or immunologic interventions that counteract such damaging effects might be a direct approach to arresting the atherogenic process. As another example, if endothelial cell modification of LDL is involved in the atherogenic process, it might be possible to show it by appropriate use of antioxidants since the modification depends on oxidative damage to the LDL molecule. The possibility that free radicals are involved in atherosclerosis has been proposed previously, particularly by Harman, but very little direct evidence is as yet available to support the hypothesis. Further research is certainly warranted. As a final example, if the ability of the macrophage to "rid itself" of lipids could be enhanced, one might be able to defer the development of clinically significant atherosclerosis by such means. As we learn more about the macrophage events involved in lipid metabolism, we may develop ways of altering their activities in the proper direction and test the hypothesis.

Finally, the available evidence that hyperlipoproteinemia is a major risk factor in human atherosclerosis seems to be overwhelming. In addition to the large body of experimental, biochemical, epidemiologic, and intervention data, we are now beginning to develop a rational and testable set of hypotheses regarding the mechanisms linking hyperlipoproteinemia to atherogenesis.

Stanford, CA

Diet and Prevention of Coronary Heart Disease and Cancer, edited by B. Halpern *et al.*, Raven Press, New York, 1986, pp. 95–111

Other support: National Heart, Lung, and Blood Institute.

From the Division of Endocrinology and Metabolism, Department of Medicine, University of California at San Diego, La Jolla.

BINDING OF COAGULATION FACTOR XI TO WASHED HUMAN PLATELETS

The binding of human coagulation factor XI to washed human platelets was studied in the presence of zinc ions, calcium ions, and high molecular weight kininogen. Saturable binding of factor XI occurred at physiological levels of these factors when high molecular weight kininogen was present. Binding required platelet stimulation and was specific, reversible, and saturable. Scatchard analysis of the binding data revealed approximately 1500 binding sites per platelet with an apparent dissociation constant of approximately 10 nM. Since this concentration of factor XI is [illegible] platelets [illegible] nM, these suggest that unstimulated factor XI [illegible] stimulated platelets

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